Supplementary material

Methods

The subjects included in this study were patients who underwent pre-operative screening before bariatric surgery in the Sint Franciscus Gasthuis in Rotterdam, the Netherlands from September 2009 to April 2011. Eligibility criteria for bariatric surgery were: age between 18 and 60 years old, body mass index (BMI) $\geq$ 35 kg/m^2. We excluded people who (a) were older than 50 years of age or; (b) had a history of smoking more than 10 cigarettes a day, or were currently smoking more than 10 cigarettes a day (with the aim to decrease the risk of including subjects with chronic obstructive pulmonary disease [COPD]); (c) were taking oral corticosteroid therapy; (d) had an asthma exacerbation four weeks before screening; (e) were unable to perform pulmonary function tests; or (f) had pulmonary disease other than asthma.

All subjects underwent baseline physical examinations including routine assessment of anthropometry and blood pressure and collection of blood samples. Waist circumference was measured directly to the body surface midway between the lower rib margin and the ileac crest. Fat free mass and fat weight (in kg and % body weight) were measured using bio-electrical impedance analysis (Bodystat 1500, Bodystat Ltd, British Isles).

Percentage excess weight loss (%EWL) was calculated as: (BMI baseline – BMI 12 months follow up) / (BMI baseline – 25) * 100.

Pulmonary function tests

Spirometry was performed with Vmax spirometer (Vmax SensorMedics Viasys, type Encore 20/22/229/62 Encore, Cardinal Health, USA) before and after 400 µg of inhaled salbutamol, according to the American Thoracic Society / European Respiratory Society guidelines[20, 21]. Static lung volumes were measured by body plethysmography, directly after the spirometry. If applicable, subjects were asked not to use longacting β2-agonists for 48 h, short-acting β2-agonists for 8 h and anti-histamines or anti-leukotriene medication 72 h before lung function

testing. Subjects who were using inhaled corticosteroids (ICS), were asked to discontinue them until bariatric surgery. Daily symptom diary and daily peak flow rates were used to screen asthma control. Subjects were permitted to use short-acting bronchodilators as rescue medication. After six weeks they returned for their second visit, during which exhaled Nitric Oxide (FeNO)(Niox mino Aerocrine, Sweden)[22], impulse oscillometry (IOS) (Masterscreen IOS system, Erich Jaeger Co., Würzburg, Germany), diffusion capacity (intrabreath method, corrected for hemoglobin and alveolar volume)[23] and methacholine provocation testing (five breath dosimeter method)[24, 25] were performed. Bronchial responsiveness to methacholine was expressed as the provocative dose of methacholine inducing a 20% fall in FEV_1 (PD_{20}). A PD_{20} < 1.8 mg was considered as a positive provocation test. If the methacholine provocation test was negative, a second provocation test was performed six weeks later. If the methacholine provocation test was negative, a second provocation test was performed six weeks later. The use of ICS was allowed during follow up.

At 3 and 6 month follow-up FEV_1 was measured by methacholine provocation testing. At 12-month follow-up two visits were scheduled. During the first visit, spirometry and body plethysmography were performed. During the second visit, Fe_{NO}, IOS, diffusion capacity and methacholine provocation testing were performed.

Questionnaires and comorbidities

Based on questionnaires the subjects with asthma were grouped into child onset of asthma if they have had the diagnosis asthma before the age of 18, and adult onset of asthma from 18 years or older. Asthma symptoms were assessed by the mini Asthma Quality of Life Questionnaire (AQLQ)[26] and the Asthma Control Questionnaire (ACQ)[27] to assess asthma complaints. An delta of 0.5 in either AQLQ or ACQ was considered clinically significant. The Epworth Sleepiness Scale[28] questionnaire was used to assess OSAS, and the GERD-Questionnaire for gastro-esophageal reflux disease (GERD)[29]. The average of 7 days data

collected with an activity meter was used to determine the total number of steps taken a day, as a measure of activity. Metabolic syndrome was diagnosed according to the National Cholesterol Education Program's Adult Treatment Panel III report (NCEP ATP-III) criteria when ≥3 of the following 5 risk factors were present: abdominal obesity, an elevated level of serum triglycerides, low serum level of high-density lipoprotein cholesterol (HDL-C), elevated blood pressure, and high serum glucose level or treatment for any of these disorders[30].

Atopy

Atopy was defined as either a positive skin-prick test (SPT) or a positive serum inhalation screen. The skin-prick tests (SPT) consisted of a battery of common aeroallergens: house-dust mite; dog, cat, and horse dander; *Aspergillus fumigatus*; mugwort; and birch and grass pollen (Vivodiagnost; ALK Benelux BV, Groningen, The Netherlands). A positive SPT was defined as at least one reaction to the aforementioned allergens as compared to the histamine positive control and saline solution-negative control. Total IgE and specific serum IgE were determined with a solid-phase two-step chemiluminescent immunoassay on the Immulite 2000 (Siemens, Los Angeles, CA). A positive serum inhalation screen was defined as at least one increased amount of specific IgE for fungus, house-dust mite, cat, dog, grass, birch or herbs.

Laboratory

Laboratory measurements were performed according to standard procedures by our Department of Clinical Chemistry. Plasma-cholesterol, HDL-cholesterol, glucose, and triglycerides, were measured using LX-20 and DxC analyzers (Beckman Coulter, Miami, FL, USA). LDL-cholesterol was calculated using the Friedewald formula. Blood cell counts and 5-part leukocyte differentiation were determined automatically using LH750 or DxH800 analyzers (Beckman Coulter). Vitamin D was determined by radioimmunoassay or chemiluminescence (LIA) on Liason analyzers (DiaSorin, Stillwater, MN, USA). Serum

markers of systemic inflammation were assessed using the Meso Scale Discovery Platform (Meso Scale Discovery, Gaitherburg, MD), for IL-6, IL-8, high-sensitivity (hs)-CRP, TNFα, GM-CSF, leptin and adiponectin. Lower limit or upper limit of detection were respectively 0.7-2500 pg/ml, 0.6-2500 pg/ml, 0.05-142 ng/ml, 0.8-2500 pg/ml, 0.61-2500 pg/ml, 0.2-100 ng/ml and 0.064-1000 ng/ml. All values which were below or above the lower or upper limit of detection of the assay, were arbitrarily set at these limits.

Results

In the BS+A group median BMI decreased from 45.1 kg/m^2 to 35.9 kg/m^2 at 3-month follow-up, 33.0 kg/m^2 at 6-month follow-up and 30.2 kg/m^2 at 12-month follow-up ($p<0.001$ for all comparisons). In the BS-A group median BMI decreased from 43.1 kg/m^2 to 36.0 kg/m^2 at 3-month follow-up, 32.3 kg/m^2 at 6-month follow-up and 29.0 kg/m^2 at 12-months follow-up ($p<0.001$ for all comparisons). The BMI was stable in the NBS+A group (35.6 kg/m^2 at baseline, 35.3 kg/m^2 at 3-month follow-up, 35.6 kg/m^2 at 6-month follow-up and 36.2 kg/m^2 at 12-month follow-up).

Also the median abdominal circumference improved in the BS+A group (133 cm baseline, 99cm 12-month follow-up; $p<0.001$) as well as in the BS-A group (127 cm baseline, 93 cm 12-month follow-up; $p<0.001$), but not in the NBS+A group (119 cm baseline, 113 cm 12-month follow-up).

AQLQ improved clinically and statistically significantly at 12-month follow-up as compared to baseline for the BS+A group (median 5.6 to 6.7, $p= 0.002$), and BS-A group (median 6.3 to 6.9, $p<0.001$). There was no significant improvement in the NBS+A group (median 5.5 to 5.8, $p=0.075$). ACQ improved clinically and statistically significantly at 12-month follow-up as compared to baseline for BS+A group (median 1.2 to 0.4, $p= 0.001$), and the NBS+A group (1.7 to 1.0, $p= 0.012$), and only statistically significantly in the BS-A group (median 0.3 to 0.0, $p= 0.001$). While there was a significant difference between BS+A and BS-A group at

baseline (p=0.001), there was no difference between these group at 12-month follow-up

(p=0.057).

Table S1 B Demographics of the study population

	NBS+A	BS+A	BS-A	p Value
Bio-impedance				
Fat free Mass	56.3 (42.9-7-9)	61.2 (47.8-100.5)	62.0 (47.2-74.7)	0.103
Fat weight (%)	43.5 (24.5-59.5)	50.9 (37.6-70.4)	50.6 (31.1-59.1)	0.124
Fat weight (kg)	45.1 (27.6-79.0)	68.1 (44.5-134.4)	62.3 (32.0-100.0)	0.046
Asthma				
Medication use at inclusion study				
Short acting bronchodilator	35.7%	41.4%	25.0%	0.367
Long acting bronchodilator	14.3%	3.4%	2.3%	0.153
Antileukotrienes	35.7%	0%	2.3%	<0.001
B_2 sympaticomimetica/ ICS	71.4%	20.7%	6.8%	<0.001
Inhaled corticosteroids	21.4%	17.2%	6.8%	0.227
Antihistamines	23.1%	24.1%	13.6%	0.447
Nasal corticosteroids	71.4%	17.2%	11.4%	<0.001

Age of onset of asthma (% as child)	75%	33%		
Atopy				
Positive inhalation screen	Not done	69.2%	42.4%	0.049
Skin prick test (% ≥1 positive wheal)	78.6%	55.2%	31.8%	0.005
IgE (kU/L)	90.7 (5.8-2026.0)	213 (5.0-2329)	52 (1.4-761.0)	0.041
Comorbidities				
Epworth Sleepiness Scale	2 (0-16)	2 (0-8)	2 (0-9)	0.964
GERD-questionnaire	6 (4-12)	6 (4-12)	6 (2-14)	0.296
Steps a day	7191 (3307-9587)	4964 (2021-12176)	4613 (2061-10083)	0.155
Metabolic syndrome	27.3%	59.3%	53.8%	0.206
Laboratory[1]				
Cholesterol (mmol/L)	4.3 (2.9-5.8)	4.7 (3.4-7.4)	5.0 (3.0-6.9)	0.570
HDL-cholesterol (mmol/L)	1.1 (0.8-1.7)	1.1 (0.7-2.3)	1.2 (0.7-2.1)	0.725
LDL-cholesterol (mmol/L)	2.7 (1.0-3.6)	2.9 (1.8-5.1)	3.0 (1.6-4.8)	0.634

Triglyceride (mmol/L)	1.2 (0.8-2.4)	1.7 (0.6-3.3)	1.4 (0.5-5.1)	0.080
Glucose (mmol/L)	5.2 (4.2-9.4)	5.6 (4.6-9.5)	5.9 (4.0-27.1)	0.029
Peripheral blood count				
Leukocytes (10^9 /L)	9.2 (4.6-12.3)	8.7 (5.3-13.1)	7.2 (4.6-11.9)	0.057
Neutrophils (%)	59 (37-79)	61 (45-72)	59 (46-70)	0.144
Lymphocytes (%)	31 (15-48)	28 (15-45)	31 (20-47)	0.282
Monocytes (%)	7 (4-14)	7 (5-13)	6 (4-13)	0.837
Eosinophils (%)	2 (0-7)	2 (0-9)	2 (0-6)	0.965
Basophils (%)	0 (0-1)	0 (0-1)	0 (0-2)	0.814

Data are presented as median (min-max).
[1] non fasting blood

BS+A,bariatric surgery and asthma group; BS-A, bariatric surgery without asthma group; ICS, inhaled corticosteroid; NBS+A, no bariatric surgery and asthma group.

Table S4 Submucosal cell counts of bronchial biopsies, before and 12 months after bariatric surgery

	BS+A			**BS-A**		
	Baseline	**12-month FU**	**p Value**	**Baseline**	**12-month FU**	**p Value**
Eosinophils (EG2)	3.0 (0.04-20.2)	1.4 (0.04-133.2)	0.889	1.3 (0.03-27.1)	2.5 (0.04-42.7)	0.826
Neutrophils (NE)	54 (11-273)	111 (24-361)	0.208	47 (0.1-139)	71 (3-228)	0.221
Mast cells (AA1)	118 (75-236)	61 (6-248)	0.036	152 (84-262)	145 (24-213)	0.125
Macrophages (CD68)	271 (67-520)	151 (65-373)	0.161	191 (7-531)	208 (58-569)	0.570
B cells (CD20)	28 (5-243)	45 (0.1-82)	1.000	36 (0.1-402)	26 (0.1-205)	0.570
T cells (CD8)	945 (321-2304)	566 (55-965)	0.050	1040 (0.2-2377)	493 (94-2641)	0.053
T cells (CD4)	510 (24-2393)	241 (6-1013)	0.161	434 (0.07-1136)	186 (0.04-719)	0.211
T cells (CD3)	1000 (161-3433)	732 (164-1165)	0.401	844 (459-2193)	559 (43-1753)	0.015

Bronchial submucosal cell count in morbidly obese subjects before and 12 months after bariatric surgery, with (BS+A, n=8) and without asthma (BS-A, n=14). p Values were calculated for the comparison between baseline and 12 months follow up within each group. Data are presented as the median (min-max), cells/mm^2 submucosa.